

Adaptable Defense: A Nudibranch Mucus Inhibits Nematocyst Discharge and Changes With Prey Type

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Abstract. Nudibranchs that feed on cnidarians must defend themselves from the prey's nematocysts or risk their own injury or death. While a nudibranch's mucus has been thought to protect the animal from nematocyst discharge, an inhibition of discharge by nudibranch mucus has never been shown. The current study investigated whether mucus from the aeolid nudibranch *Aeolidia papillosa* would inhibit nematocyst discharge from four species of sea anemone prey. Sea anemone tentacles were contacted with mucus-coated gelatin probes, and nematocyst discharge was quantified and compared with control probes of gelatin only. Mucus from *A. papillosa* inhibited the discharge of nematocysts from sea anemone tentacles. This inhibition was specifically limited to the anemone species on which the nudibranch had been feeding. When the prey species was changed, the mucus changed within 2 weeks to inhibit the nematocyst discharge of the new prey species. The nudibranchs apparently produce the inhibitory mucus rather than simply becoming coated in anemone mucus during feeding. Because of the intimate association between most aeolid nudibranchs and their prey, an adaptable mucus protection could have a significant impact on the behavior, distribution, and life history of the nudibranchs.

Introduction

All predators must overcome their prey's defenses to feed. In the case of nudibranchs that feed on cnidarians, the predator must defend itself from the prey's nematocysts or risk its own injury or death (Harris, 1973; Conklin and Mariscal, 1977). Nematocysts from non-prey cnidarians can kill the nudibranch (Grosvenor, 1903); even the prey species can be dangerous if the individual is large enough (Harris,

1973, 1986; Conklin and Mariscal, 1977). Defenses that might protect aeolids from nematocysts include behaviors that limit contact of the nudibranch with the prey (Grosvenor, 1903), morphological adaptations such as ellipsoid vacuolate cells of the epithelium (Graham, 1938; Porter and Rivera, 1980; Martin and Walther, 2003) or a cuticle that lines the mouth region of the nudibranch (Edmunds, 1966), and copious mucous secretions (Graham, 1938; Russell, 1942; Edmunds, 1966).

It has been hypothesized for over a century that the nudibranch's mucus serves as a protective barrier against nematocysts (Boutan, 1898). Grosvenor (1903) suggested that there might be some acclimatization to nematocysts as part of the mucous defense, because copious mucus was not enough to protect nudibranchs from different cnidarian species. However, Salvini-Plawen (1972) hypothesized that "hyper-viscous" mucous secretions would inhibit nematocyst discharge generally and that it would be unnecessary to adapt the protective nature of mucus to specific prey. Conklin and Mariscal (1977) noted that the aeolid nudibranch *Spurilla neapolitana* was apparently stung by its prey anemones during the first few minutes of feeding but the nudibranchs' behavior indicated that nematocyst discharge then ceased. These authors hypothesized that aeolids might produce or acquire (from the prey) a substance that prevented nematocyst discharge. Recently Mauch and Elliott (1997) found that mucus from the aeolid nudibranch *Aeolidia papillosa* caused fewer nematocysts to discharge from its potential prey, the sea anemone *Anthopleura elegantissima*, than mucus from other gastropods did. Mauch and Elliott (1997) hypothesized that the nudibranchs might adapt their mucus as protection from their cnidarian prey, but the nudibranch mucus was tested on only one species of cnidarian.

While a few nudibranchs are highly specialized and feed exclusively on one prey species, most nudibranch species

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can feed on several different prey species (Thompson, 1976; Todd *et al.*, 2001). Monophagous species might have defensive mucus that works well against the nematocysts of a single prey species, but the more generalist feeders need an effective defense against a number of cnidarian species (with different nematocyst types). Such nudibranchs might require defensive mucus that changes properties depending upon which prey is being consumed.

The current study investigated whether mucus from the aeolid nudibranch *Aeolidia papillosa* inhibited nematocyst discharge from different sea anemone species, and whether the inhibitory nature of the mucus changed when the prey species was changed. Individuals of *A. papillosa* feed almost exclusively on sea anemones (Hall and Todd, 1986). In its New England subtidal habitat, *A. papillosa* is frequently found among populations of the sea anemone *Metridium senile*, but is also found associated with a variety of other sea anemone species (Harris, 1973; Reidy, 1996). Individuals of *A. papillosa* have been induced to change prey species in the laboratory (Hall *et al.*, 1982; Hall and Todd, 1984, 1986), but in the field a single prey species may dominate a large area. Concentrated prey distribution and low nudibranch mobility means that individual nudibranchs might spend most of their lives closely associated with just one prey species (Harris, 1973, 1987; Todd, 1983). Because of this intimate association between *A. papillosa* and its prey, protection from the prey's nematocysts has a significant impact on the behavior, distribution, and life history of the nudibranch.

We used mucus-coated gelatin probes in a highly reproducible method (Watson and Hudson, 1994) to investigate the effects of *A. papillosa* mucus on nematocyst discharge. When gelatin probes are touched to sea anemone tentacles, nematocysts that discharge into the gelatin remain attached to the probe and are easily counted (Watson and Hessinger, 1989; Watson *et al.*, 1999). In this study, mucus-coated gelatin probes were used to mimic the contact of a nudibranch to the sea anemones. We also probed sea anemones in the presence of N-acetylneuraminic acid (NANA). NANA, which mimics the complex molecules containing N-acetylated sugars commonly found on the sea anemones' normal prey (Thorington and Hessinger, 1988), has been found to increase the sensitivity of sea anemone nematocytes to mechanical stimulation (Thorington and Hessinger, 1988, 1990, 1998; Watson and Hessinger, 1994). With NANA, we can therefore determine whether nudibranch mucus actually inhibits nematocyst discharge or simply provides no stimulus for discharge.

Materials and Methods

Animal collection and maintenance

Individuals of the nudibranch *Aeolidia papillosa* and the sea anemone *Metridium senile* were collected from a float-

ing commercial fishing dock in Portsmouth, New Hampshire. Individuals of *A. papillosa* and the sea anemones *Urticina felina* and *Aulactinia stella* were collected midway through the littoral zone on bedrock at west Quoddy Head in Lubec, Maine (courtesy of C. Sisson). Twelve individuals of *A. papillosa*, living among individuals of the sea anemone *Anthopleura elegantissima*, were collected from the low intertidal zone on the coast of California (supplied by Pacific Biomarine Supply, Venice, CA). Individuals of *A. elegantissima* were collected from the low intertidal zone of San Juan Island, Washington (courtesy of D. Duggins). All animals were kept in refrigerated aquaria with recirculating and refiltered seawater (11 °C, 31‰ salinity, pH 8.2) collected at the Darling Laboratory, University of Maine, Wapole, Maine. Anemones were kept in separate plastic or glass containers within the aquaria, and the nudibranchs were placed in separate acrylic plastic containers, segregated according to the sea anemone species on which they were feeding. Sea anemones were fed frozen brine shrimp every 2 days, and the nudibranchs were fed sea anemones *ad libitum*. The sea anemones that were used as prey all had pedal disk diameters less than 3 cm, and the nudibranchs ranged in length from 1 cm to 7 cm. Individuals of *M. senile* were held for no more than 2 months before being replaced with other animals; the other species of sea anemones were held for less than a month.

Control probes, mucus-coated probes, and general probing of sea anemone tentacles

We used gelatin probes to quantify nematocyst discharge from tentacles of sea anemones. Each probe was made by coating one end of a 6-cm length of monofilament fishing line (Stren, 6-lb-test or 17-lb-test) with 25% (weight/volume) gelatin in E-pure deionized water (modified slightly from Watson and Hudson, 1994). Occasionally, the gelatin did not adhere well to the probe, and such probes were discarded. The gelatin adhered better to the 17-lb-test line than to the 6-lb-test line, so the number of probes discarded was smaller when the heavier line was used, as it indeed was, in later experiments. Probes that were coated with only gelatin served as controls.

To prepare an experimental mucus-coated probe, a nudibranch was removed from the water and a gelatin probe was gently wiped across its dorsal surface (from anterior to posterior) four times. Three mucus-coated probes were made from each nudibranch. Anemones that had not been fed for between 24 and 36 hs were placed into separate glass dishes that contained a probing solution of either filtered seawater alone, or filtered seawater and NANA. Ten minutes after the anemones were placed into the probing solution, each dish was placed under an Olympus SC30 stereomicroscope, and probing began with the edge of the probe tip being lightly touched to one tentacle about 5 mm

proximal to the tentacle tip. Three control probes and three experimental probes, all prepared from the same nudibranch, were used to probe each anemone. Used probes were fixed in 2.5% glutaraldehyde in filtered seawater for at least 30 s and then placed into 3 drops of deionized water on a microscope slide. Individual nematocysts that had discharged into the gelatin were counted, using an Olympus CK2 inverted phase contrast microscope equipped with a 40 \times objective lens. In early experiments with probes made from 6-lb-test monofilament line, we counted nematocyst capsules from at least three fields of view, and the mean was calculated for each probe. In later experiments, when we used probes made from 17-lb-test line, the nematocyst capsules from one central field of view were counted. Using these techniques, between 75% and 100% of the discharged nematocysts on each probe were counted, and both types of probes yielded highly reproducible results.

Does a nudibranch's mucus inhibit the nematocyst discharge from the tentacles of its prey species?

Probes were coated with mucus from 12 individuals of *Aeolidia papillosa* of mixed sizes that had been feeding on the sea anemone *Metridium senile* and with mucus from 6 individuals of *A. papillosa* of mixed sizes that had been feeding on the sea anemone *Urticina felina*. These mucus-coated probes were then used to probe feeding tentacles from the sea anemones *M. senile*, *U. felina*, and *Aulactinia stella*. In each experiment, one anemone was always tested with three control probes and then, immediately afterwards, with three experimental (mucus-coated) probes; the experimental probes were coated with mucus from an individual nudibranch. The mean number of discharged nematocysts in the three probes was used to calculate a grand mean of nematocyst discharge in response to mucus from each nudibranch. These grand means were tested for normality and equal variances and then analyzed using Student's *t* tests (or the Mann-Whitney *U* test when variances were unequal) to compare nematocyst discharge into mucus-coated probes with discharge into control probes for each species. To minimize the effects of captivity on nematocyst discharge, each species of anemone was tested within 2 weeks of its collection.

If the prey species is changed, will the effect of the nudibranch mucus on nematocyst discharge also change?

Seven specimens of *A. papillosa* were collected from several New England localities and were fed *U. felina* for 16 days in the laboratory. During that period, mucus-coated probes were used to test nematocyst discharge from the tentacles of *U. felina* and *M. senile*. The nudibranchs' prey was then switched from *U. felina* to *M. senile* and, over a 2-week interval, mucus-coated probes were used periodically to test nematocyst discharge from both sea anemone

species. Because the baseline number of nematocysts differed between the two sea anemone species, these data were converted to relative values, where a relative value of 1.0 represents the average number of nematocysts discharged into control probes. Data collected from mucus-coated probes for each day were converted to an average relative discharge based on controls for that day from the same anemones. Results from tests on each sea anemone species were analyzed by ANOVA and with Scheffé's test for pairwise comparisons.

If a second prey species is offered, will the effect of the nudibranch mucus on nematocyst discharge change?

Four individuals of *A. papillosa* were fed the sea anemone *M. senile* for 21 days. Mucus-coated probes were used to test nematocyst discharge from feeding tentacles of *M. senile* and the sea anemone *Anthopleura elegantissima*. The nudibranchs were then offered both *M. senile* and *Anthopleura elegantissima*. *Anthopleura elegantissima* is not found on the coast of New England, so the experimental nudibranchs could not have encountered this prey species before. Fourteen days later, mucus-coated probes were used to test nematocyst discharge from the tentacles of both species of prey anemone. Data were converted to relative discharge values as described for the previous experiment and analyzed using Student's *t* tests.

Does the nudibranch produce its own inhibitory mucus or simply become covered in the prey's mucus?

Any inhibitory effect of the nudibranch's mucus coating could be a result of the nudibranch being coated in the mucus of the prey species during feeding. To test this possibility, we compared the inhibitory effectiveness of mucus removed from a particular region of a nudibranch with mucus removed from that same region 45 min after wiping that area clean. Probes were coated with mucus from eight individuals of *A. papillosa* of mixed sizes that had been feeding on the sea anemone *M. senile*. The probes were coated with mucus from the dorsal surface of the nudibranch immediately behind the heart. Each nudibranch was then transferred to fresh filtered seawater. Sterile cotton swabs were used to wipe the mucus from the dorsal surface of the nudibranch and from the surrounding cerata. Six additional swabs were used to wipe the nudibranch a total of seven times, and the nudibranch was again transferred to fresh filtered seawater. After the nudibranch was allowed to recover from this treatment for 45 min, additional probes were coated with mucus from its dorsal surface immediately behind the heart. The mucus-coated probes were then all used to probe feeding tentacles of *M. senile*. Three probes were coated with mucus from each nudibranch for each treatment (unswabbed and swabbed). Counting of all probes was done blind. The mean number of discharged nemato-

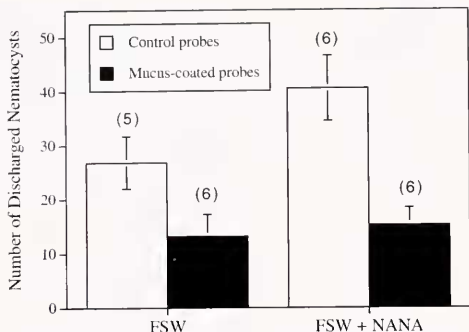


Figure 1. Number of nematocysts discharged into control probes or into probes coated with mucus from individuals of *Aeolidia papillosa* that had been feeding on *Metridium senile*. Mucus-coated probes were used to probe tentacles of the sea anemone *M. senile* in both filtered seawater (FSW) and FSW containing 10^{-7} M N-acetylneuraminic acid (FSW + NANA), and compared against control probes of gelatin only. The numbers in parentheses are the numbers of anemones used (control probes) or the numbers of nudibranchs used (mucus-coated probes) per test.

cysts into mucus-coated probes from unswabbed nudibranchs was compared to nematocyst discharge into mucus-coated probes from swabbed nudibranchs and into control probes. Analysis was done by ANOVA and with Scheffé's test for pairwise comparisons.

We also investigated whether the mucus of *M. senile* individuals inhibited discharge from other individuals of *M. senile* held in separate containers. Individual sea anemones were transferred to filtered seawater, allowed to recover for 10 min, transferred to fresh filtered seawater again, allowed to recover for 10 min, and finally transferred to a dry, clean glass dish. The sea anemone secreted mucus for 10 min and was removed from the dish. The mucus was removed from the dish and stored in sterile microcentrifuge tubes. Gelatin probes were placed into the mucus for 2 min and used to probe different individuals of *M. senile*. Three probes were coated with mucus from each of four anemones. The mean number of discharged nematocysts in the mucus-coated probes was compared to that number in control probes with no mucus. Data were analyzed using Student's *t* tests as described above.

Results

Does a nudibranch's mucus inhibit the nematocyst discharge from the tentacles of its prey species?

When tentacles of the sea anemone *Metridium senile* were probed with control probes in seawater, a baseline discharge response was observed (Fig. 1). The nematocysts that discharged into the gelatin were mostly basitrichous isorhizas along with some microbasal p-mastigophores.

Gelatin probes coated with mucus from the nudibranch *Aeolidia papillosa* that had been feeding on *M. senile* elicited 51% fewer nematocysts to discharge from *M. senile* than control probes did (Student's *t* test, $t_0 = 5.1$, $P = 0.0006$) (Fig. 1). When probing was done in seawater containing 10^{-7} M NANA, the number of nematocysts that discharged into mucus-coated probes was almost 65% less than the number that discharged into control probes (Student's *t* test, $t_{10} = 9.1$, $P < 0.0001$) (Fig. 1), but the mean number of discharges into control probes was higher than when NANA was omitted (Student's *t* test, $t_9 = 4.1$, $P < 0.003$). For each anemone species, probing in 10^{-7} M NANA elicited a greater number of discharged nematocysts than probing in seawater alone (Fig. 2). Statistical results were as follows: for *M. senile*, Student's *t* test, $t_9 = 4.1$, $P < 0.003$; for *Aulactinia stella*, Student's *t* test, $t_2 = 4.5$, $P < 0.05$; for *Anthopleura elegantissima*, Student's *t* test, $t_4 = 5.0$, $P = 0.008$. Because it increased the response, 10^{-7} M NANA was used for all subsequent experiments.

When probes coated with mucus from *A. papillosa* that had been feeding on *M. senile* were used to probe the sea anemones *Urticina felina* and *Aulactinia stella*, there was a small but nonsignificant increase in nematocyst discharge over controls (for *U. felina*, Student's *t* test, $t_{10} = 0.394$, $P = 0.70$; for *Aulactinia stella*, Student's *t* test, $t_{26} = 0.76$, $P = 0.46$) (Fig. 3). As before, significantly fewer nematocysts discharged into mucus-coated probes from tentacles of *M. senile* than into control probes (Student's *t* test, $t_8 = 9.0$, $P < 0.0001$) (Fig. 3). Nematocysts from *U. felina* were mostly microbasal p-mastigophores and some basitrichous isorhizas. Nematocysts from *Aulactinia stella* were basitrichous isorhizas and microbasal p-mastigophores in about equal numbers. The mucus effectiveness was sim-

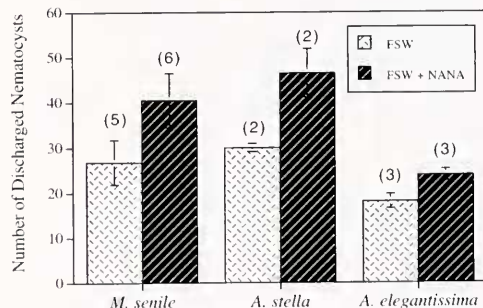


Figure 2. Number of nematocysts discharged into control probes. Tentacles of the sea anemones *Metridium senile*, *Aulactinia stella*, and *Anthopleura elegantissima* were probed in filtered seawater (FSW) and tentacles of other individuals of each species were probed in FSW containing 10^{-7} M N-acetylneuraminic acid (FSW + NANA). The numbers in parentheses are the numbers of anemones used per test.

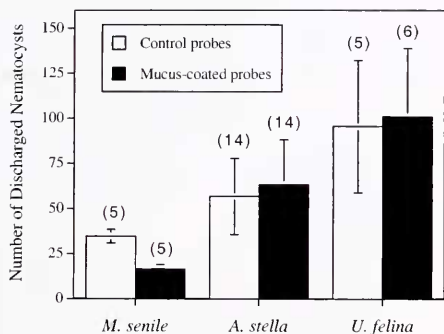


Figure 3. Number of nematocysts discharged into control probes or into probes coated with mucus from individuals of *Aeolidia papillosa* that had been feeding on *Metridium senile* (\pm SD). Mucus-coated probes were used to probe tentacles of the sea anemones *M. senile*, *Aulactinia stella*, and *Urticina felina* (in 10^{-7} M N-acetylneuraminic acid) and were compared against control probes of gelatin only. The numbers in parentheses are the numbers of anemones used (control probes) or the numbers of nudibranchs used (mucus-coated probes) per test.

ilar among individual nudibranchs regardless of their size (ANOVA, $F_{6,48} = 0.76$, $P = 0.61$).

When gelatin probes coated with mucus from individuals of *A. papillosa* that had been feeding upon *U. felina* were tested on the tentacles of *U. felina*, nematocyst discharge was 67% less than discharge into the control probes (Mann-Whitney *U* test, $U_{4,6} = 24$, $P = 0.01$) (Fig. 4). When probes coated with mucus from *A. papillosa* that had been feeding on *U. felina* were used to probe the sea anemones *M. senile* and *Aulactinia stella*, nematocyst discharge was

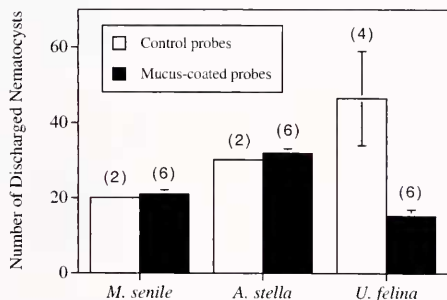


Figure 4. Number of nematocysts discharged into control probes or into probes coated with mucus from individuals of *Aeolidia papillosa* that had been feeding on *Urticina felina* (\pm SD). Mucus-coated probes were used to probe tentacles of the sea anemones *Metridium senile*, *Aulactinia stella*, and *U. felina* (in 10^{-7} M N-acetylneuraminic acid) and were compared against control probes of gelatin only. The numbers in parentheses are the numbers of anemones used (control probes) or the numbers of nudibranchs used (mucus-coated probes) per test.

no different than in controls (for *M. senile*, Student's *t* test, $t_6 = 1.10$, $P = 0.31$; for *Aulactinia stella*, Student's *t* test, $t_6 = 1.96$, $P = 0.098$) (Fig. 4).

If the prey species is changed, will the effect of the nudibranch mucus on nematocyst discharge also change?

Seven individuals of *A. papillosa* that had been collected from several different New England localities were fed *U. felina* for 16 days in the laboratory. As before, nematocyst discharge into mucus-coated probes was 50% less than nematocyst discharge into control probes for *U. felina*, but not for *M. senile* (Fig. 5). The nudibranchs' prey was then switched from *U. felina* to *M. senile*, and over a period of 2 weeks, the mucus from the nudibranchs was tested periodically against both sea anemone species. Over the course of the experiment, the number of nematocysts that discharged into mucus-coated probes increased when *M. senile* was probed (ANOVA, $F_{3,21} = 8.20$, $P = 0.0004$) and decreased when *U. felina* was probed (ANOVA, $F_{3,19} = 23.12$, $P < 0.0001$). Within 10 days after the prey switch, touching *M. senile* tentacles with mucus-coated probes elicited the discharge of 75% fewer nematocysts than control probes did (Scheffé's test, $P = 0.0067$) (Fig. 5). By the end of the experiment, touching *U. felina* tentacles with mucus-coated probes again resulted in a small but nonsignificant

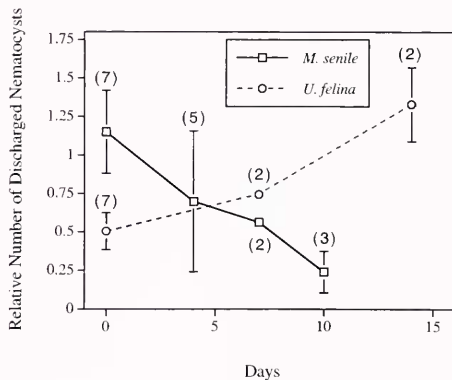


Figure 5. The effect of *Aeolidia papillosa* mucus on the relative number of discharged nematocysts following prey switch from the sea anemone *Urticina felina* to the sea anemone *Metridium senile* (\pm SD). Mucus-coated probes were used to probe tentacles of the sea anemones *U. felina* and *M. senile* (in 10^{-7} M N-acetylneuraminic acid), and were compared against controls for each species for a period of 2 weeks. A relative value of 1.0 corresponds to the number of nematocysts discharged into control probes on Day 0 for each sea anemone species. Data obtained from mucus-coated probes for each day were normalized against controls for that day and for each species of sea anemone. The numbers in parentheses are the numbers of nudibranchs used per test.

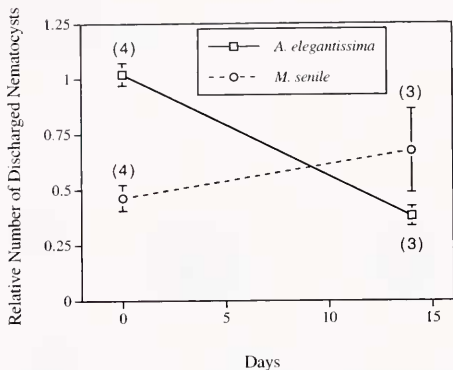


Figure 6. The effect of *Aeolidia papillosa* mucus on the relative number of discharged nematocysts following prey switch from the sea anemone *Metridium senile* only to both *M. senile* and the sea anemone *Anthopleura elegantissima*. Mucus-coated probes were used to probe tentacles of the sea anemones *M. senile* and *A. elegantissima* (in 10^{-7} M N-acetylneuraminic acid), and were compared against controls for each species after 2 weeks. A relative value of 1.0 corresponds to the number of nematocysts discharged into control probes on Day 0 for each sea anemone species. Data obtained from mucus-coated probes for each day were normalized against controls for that day and for each species of sea anemone. The numbers in parentheses are the numbers of nudibranchs used per test.

increase in nematocyst discharge over that into control probes (Scheffé's test, $P = 0.072$) (Fig. 5).

If a second prey species is offered, will the effect of the nudibranch mucus on nematocyst discharge change?

Four individuals of *A. papillosa* were fed *M. senile* for at least 21 days, and tentacles of both *M. senile* and *Anthopleura elegantissima* were tested with mucus-coated probes. *M. senile* nematocyst discharge was 56% lower into mucus-coated probes than into control probes (Student's t test, $t_6 = 9.6$, $P < 0.0001$); *Anthopleura elegantissima* nematocyst discharge into mucus-coated probes was not different from discharge into control probes (Student's t test, $t_6 = 0.545$, $P = 0.61$) (Fig. 6). Nematocysts from *Anthopleura elegantissima* were all basitrichous isorhizas. The nudibranchs were then offered both *M. senile* and *Anthopleura elegantissima*. After the nudibranchs had been feeding on both prey species for 14 days, testing with mucus-coated probes showed nematocyst discharges 34% lower than control probes for *M. senile* (Student's t test, $t_4 = 2.78$, $P = 0.05$) and 64% lower than control probes for *Anthopleura elegantissima* (Student's t test, $t_4 = 17.06$, $P < 0.0001$) (Fig. 6).

Does the nudibranch produce its own inhibitory mucus or simply become covered in the prey's mucus?

Mucus was wiped away from the dorsal surface of eight individuals of *A. papillosa* that had been feeding on the sea anemone *M. senile*. Forty-five min later, gelatin probes coated with new mucus from those same nudibranchs were used to probe tentacles of *M. senile*. Nematocyst discharge into those probes was no different from nematocyst discharge into probes coated with mucus from the same eight individuals of *A. papillosa* before wiping the mucus away (Scheffé's test, $P = 0.60$) (Fig. 7). Nematocyst discharge into both groups of mucus-coated probes was well under half the number that discharged into control probes (ANOVA, $F_{2,16} = 10.54$, $P = 0.001$) (Fig. 7).

When probes coated with *M. senile* mucus were used to probe tentacles of other individuals of *M. senile*, nematocysts discharged into the probes in numbers no different than into control probes (Student's t test, $t_6 = 1.14$, $P = 0.30$) (Fig. 8).

Discussion

The present study shows, for the first time, that the mucus from a nudibranch specifically inhibits the discharge of nematocysts from sea anemone tentacles. This inhibition of nematocyst discharge is limited to the anemone species on which the nudibranch has been feeding. Moreover, the nudibranch mucus changes to inhibit the nematocyst discharge of a different sea anemone species if the nudibranch

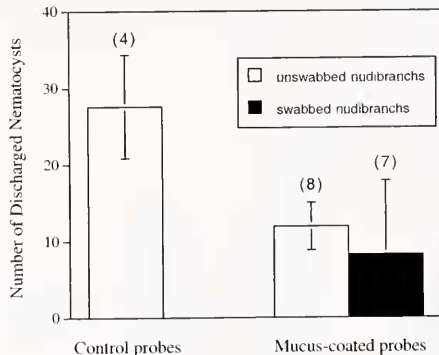


Figure 7. The number of nematocysts discharged into control probes or into probes coated with mucus from individuals of *Aeolidia papillosa* that had been feeding on *Metridium senile*. Probing of *M. senile* tentacles was done in 10^{-7} M N-acetylneuraminic acid. Mucus-coated probes were prepared both before the original mucus coating was removed from the nudibranch (unswabbed nudibranchs) and 45 min after mucus removal (swabbed nudibranchs). The numbers in parentheses are the numbers of anemones used (control probes) or the numbers of nudibranchs used (mucus-coated probes) per test.

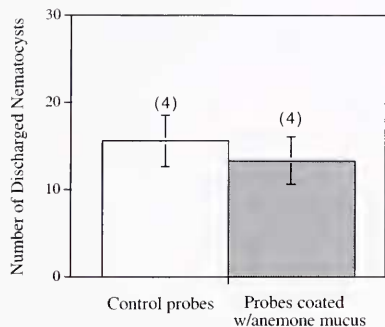


Figure 8. The number of nematocysts discharged into control probes or into probes coated with mucus from individuals of the sea anemone *Metridium senile*. Probing of *M. senile* tentacles was done in 10^{-7} M N-acetylneuraminic acid. The numbers in parentheses are the numbers of anemones used per test.

begins to feed on that new species. If nudibranchs are fed two different species of sea anemone, their mucus inhibits nematocyst discharge from both prey species. Nudibranchs produce their own inhibitory mucus and do not simply mimic sea anemones by becoming covered with anemone mucus during feeding.

Mucus from nudibranchs that had been fed the sea anemone *Metridium senile* greatly inhibited nematocyst discharge from *M. senile*, but not from *Urticina felina* or *Aulactinia stella* (Fig. 3). Likewise, mucus from nudibranchs that had been fed *U. felina* inhibited nematocyst discharge from *U. felina*, but not from *M. senile* or *Aulactinia stella* (Fig. 4). Within 10 days after the prey of *A. papillosa* was changed from *U. felina* to *M. senile*, the mucus of *A. papillosa* no longer inhibited nematocyst discharge from *U. felina*, but did inhibit nematocyst discharge from *M. senile* (Fig. 5). In another experiment, *A. papillosa* was fed both *M. senile* and *Anthopleura elegantissima*. After 2 weeks, the mucus of the nudibranch inhibited nematocyst discharge from both *M. senile* and *Anthopleura elegantissima* (Fig. 6). Overall, *A. papillosa* mucus reduced nematocyst discharge from all prey anemones by 60% below control levels ($60.3\% \pm 4.1\%$).

Mucus from nudibranchs that had been freshly swabbed of their previous mucous coating inhibited nematocyst discharge from anemone prey (Fig. 7), indicating that the nudibranchs do not simply become covered with anemone mucus. Mucus from nudibranchs held in the same aquarium with sea anemones did not inhibit nematocyst discharge from that species. This suggests that the nudibranch mucus is altered only after feeding. Whether the mucus of *A. papillosa* is altered by the nudibranch itself or if compounds acquired from the prey are subsequently incorporated into the mucus is not yet known. Nudibranchs acquire numerous

prey compounds and use them for their own defense from predators (Avila *et al.*, 1991; McClintock *et al.*, 1994), but some nudibranchs also synthesize their own chemical defenses (Cimino *et al.*, 1983; Faulkner, 1992). Because sea anemones do not sting themselves or clonemates, there might be compounds that prevent nematocyst discharge on or within the sea anemone (Pantin, 1942; Ertman and Davenport, 1981), but so far such compounds have not been identified in any cnidarians. In addition, we found in this study that mucus from individuals of one color morph of *M. senile* did not inhibit nematocyst discharge from another color morph of *M. senile* (Fig. 8), indicating that a prey species' mucus is not necessarily inhibitory to other members of that species. Therefore, the nudibranchs are not relying solely on compounds acquired from the prey's mucus. Acolid nudibranchs secrete mucus and other secretions from gland cells found in various locations on their bodies (Edmunds, 1966). Histochemical evidence suggests that most species (including *A. papillosa*) have acidic mucopolysaccharides as at least part of the mucus (Edmunds, 1966; Porter and Rivera, 1983), but other components, which are not well characterized, may be secreted from gland cells and mix with the mucus on the surface of the nudibranch (Edmunds, 1966). The mucous secretion we used to coat the gelatin probes probably includes components from any or all of these gland cells.

Most of our studies were done in the presence of N-acetylneuraminic acid (NANA), which increased the baseline number of nematocysts that discharged into the gelatin probes (Fig. 2). Because the nudibranch mucus inhibited nematocyst discharge in the presence of NANA (Fig. 1), the mucus must either reduce the mechanical stimulation caused by the nudibranch while feeding or inhibit the signal pathway leading to nematocyst discharge. When nudibranch mucus was tested against non-prey sea anemones, nematocyst discharge actually increased by a modest, but consistent, number over controls ($11.8\% \pm 4.5\%$). Therefore nudibranch mucus may actually promote nematocyst discharge in species that are not the current prey. *Acolidia papillosa* shows a strong preference for its most recent prey species (Hall *et al.*, 1982). Because the mucus of *A. papillosa* inhibits nematocyst discharge from current prey species but not from other potential prey species, one might expect the current prey species to remain the preferred prey of the nudibranch.

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